D11

71. (Amended) A cell comprising a modified bleomycin gene cluster, wherein the modified bleomycin gene cluster comprises a nucleic acid which encodes a protein comprising SEQ ID NO:115, said cell producing elevated amounts of bleomycin as compared to the wild type cell.

D12

73. (Twice amended) The cell of claim 71, wherein said cell overexpresses a resistance gene from the bleomycin gene cluster and wherein said resistance gene is a selected from the group consisting of blmA and blmB.

In accordance with the requirements of 37 C.F.R. § 1.121, a marked up version showing the changes to the claims, is attached herewith as Appendix A. For the Examiner's convenience, a complete claim set of the currently pending claims is also submitted herewith as Appendix B.

These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with any objection or rejection of record.

REMARKS

The Status of the Claims.

Claims 1-3, 9, 10, 12-14, 21, 40-45, 71 and 73 are pending with entry of this amendment, claims 5, 15, 17, and 72 being cancelled. Claims 1, 10, 12, 13, 14, 21, 40, 71 and 73 are amended herein. Claim 9 has been indicated as being allowed. Action (dated December 4, 2002) at page 16. These amendments introduce no new matter and support is replete throughout the specification.

With respect to claim 1, support for "highly" can be found throughout the specification. For example, see specification at page 10, lines 33 to page 11, line 2. Claim 10 is amended to correct typographical errors. Claims 12, 13, and 14 are amended to include SEQ ID NOs. At the request of the Examiner, Claims 21 and 71 are amended to include a limitation directed to the elected subject matter (ORF8, SEQ ID NO. 115). Claim 40 is amended to remove claims that were canceled and to correct antecedent basis. Claim 73 is amended to incorporate the limitations of the claim it depended on which is canceled herein.

Applicants submit that no new matter is added to the application by way of the above Amendment. Accordingly, entry of the Amendment is respectfully requested.

The Drawings.

The Examiner considered the drawings mailed on September 12, 2002 as being informal. Action (dated December 4, 2002) at page 3. Applicants submit herewith a Letter to the Draftsperson along with a set of Formal Figures.

Compliance with the Sequence Listing Rules.

Applicants note that the Examiner has indicated that the application is in full compliance with the sequence rules. Action (dated December 4, 2002) at page 3. See also the objection to the specification section below regarding Table II.

The Information Disclosure Statement.

Applicants note with appreciation the Examiner's thorough consideration of the references cited in the Information Disclosure Statement (Form 1449) submitted on September 12, 2002.

The Examiner indicated that a copy of the AP reference (Gaidenko et al.) was missing. In the response filed May 5, 2003, Applicants submitted an additional Form 1449, which included a copy of the reference AP of the Information Disclosure Statement 1449 form submitted on December 6, 2001. The reference is in Russian with a translation of the abstract in English at page 19 of the reference. The reference is described in the specification at page 72, lines 2-7. Applicants note that the Examiner has sent back an initialed copy of the Form 1449 filed on May 5, 2003 in the Examiner's communication dated May 29, 2003, indicating that the Examiner has reviewed the reference and that it would be placed on record in the application.

Objection to the Specification

The specification was objected to for being allegedly confusing for the following reasons.

a) Figure 6F

The Examiner alleges that a description of Figure 6F was omitted in the previous amendment (submitted on September 12, 2002). Action (dated December 4, 2002) at page 5. Applicants have amended the description of Figure 6 to include the description of Figure 6F. Support for the amendment is found in Figure 6 as originally filed. Accordingly, the objection should be withdrawn.

b) Page 19, line 14

The Examiner alleges that it is unclear "why two SEQ ID Nos are used to describe three accession numbers, particularly if, as noted by the Applicants, AF210311 pertains to ptpA which is described in the instant specification as SEQ ID NO:3." Action (dated December 4, 2002) at page 5. As Applicants indicated in the previous amendment (submitted on September 12, 2002), Accession AF210249 replaces sequence(s) AF149091. In addition, Applicants have amended the specification to clarify that AF210311 is identified as SEQ ID NO.3. See specification at page 23, lines 10-11. Accordingly, the objection should be withdrawn.

c) Table 1, on page 19

The Examiner alleges that listing the SEQ ID Nos of the amino acids would be helpful. Action (dated December 4, 2002) at page 5. As previously indicated, Applicants note that SEQ ID Numbers are not required by the rules. However, in an effort to further prosecution, Applicants have amended Table 1 to include the SEQ ID Nos. and the orf number that comprises a blm gene product. Support for the amendment is found throughout the specification, e.g., in Table II at page 21-23. As a result, the objection should be withdrawn.

d) Table 1, on page 19

The Examiner alleges that the term "RfaE" is unclear. Applicants have amended the Table 1 to clarify the term. Specifically, Applicants have referenced the accession number AAD07904, which encodes an ADP-heptose synthase (rfae). The accession number is available in GenBank using a routine search. It appears that the accession number listed in the originally filed application ("AA07904.1") had typographical errors. See originally filed Table 1, on page 19. Accordingly, the objection should be withdrawn.

Applicants also amend the row in Table I that is directed to orf26(blmX). The number of amino acids is 2162. Support for the amendment can be found in the originally filed sequence listing in SEQ ID NO.:1 marked as orf26. See originally filed SEQ ID listing at page 4-9 and SEQ ID NO.: 97 at pages 69-77 of sequence listing submitted on June 21, 2001.

e) Table II

The Examiner alleges that position numbers in Table II are unclear. Action (dated December 4, 2002) at page 3, 4, and 5. Applicants have canceled the position numbers. As a result, the objection with respect to Table II is moot. Furthermore, the position numbers were based on the originally filed sequence listing, which was replaced by the Sequence listing filed on June 21, 2001.

Unlike the originally filed sequence listing, the Sequence listing filed on June 21, 2001 independently lists each of the recited ORFs (making the position numbers in the table both unnecessary and unhelpful). The positions of the orfs are delineated in Figures 1B and 2. Accordingly, the position of the orfs is clear, the table has been amended to render the rejection moot, and the objection should be withdrawn.

f) Page 69

The Examiner alleges that the following accession numbers, AL008967, AL031107, and AL049863, are unclear because these numbers cannot be found in GenBank under protein databases. Action (dated December 4, 2002) at page 5. Applicants have amended to the specification to indicate that these can be found in the nucleotide database. All three accession numbers are available in GenBank using a routine search. Accordingly, the objection should be withdrawn.

Thus, Applicants respectfully request that the objection with respect to the specification be withdrawn.

Objections to the Claims

Claims 21, 40-45 and 71-73

Objections to claims 21, 40-45 and 71-73 were maintained for allegedly containing non-elected subject matter. Applicants have amended claims 21 and 71 to included ORF8 (the elected subject matter). Claims 40-45 depend on claim 21, thus the limitations of amended claim 21 will be incorporated into claims 40-45, and claim 73 depends on claim 71, thus the limitations of amended claim 71 will be incorporated into claim 73. Claim 72 has been canceled; thus, the objection with respect to this claim is moot. Accordingly, the objection with respect to claims 21, 40-45 and 71 and 73 should be withdrawn.

Claim 5

Claim 5 was objected to for allegedly having a period embedded within the text. Action (dated December 4, 2002) at page 14. Applicants have canceled claim 5; thus, the objection with respect to this claim is moot.

Claims 12-15

Claims 12-15 were objected to for allegedly containing references to orfs with terms like "blmVIII" without using SEQ ID Nos. Action (dated December 4, 2002) at page 14.

Applicants have amended claims 12-14 to include the SEQ ID Nos. Claim 15 has been canceled; thus, the objection with respect to this claim is moot. As a result, the objection with respect to claims 12-14 should be withdrawn.

Claim 73

Claim 73 was objected to for allegedly having a typographical error, i.e., a comma after blmA. Action (dated December 4, 2002) at page 14. Applicants have amended the claim to remove the comma. Thus, the objection with respect to claim 73 should be withdrawn.

Thus, Applicants respectfully request that the objections with respect to the claims be withdrawn.

35 U.S.C. §112, Second Paragraph.

Claims 1-3 and 40-45

Claims 1-3 and 40-45 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the term "stringent conditions" is allegedly unclear as to its metes and bounds. Action (dated December 4, 2002) at page 9. Applicants traverse. However, in order to further prosecution, Applicants have amended claim 1 (upon which claims 2-3, and 40-45 depend) to include the term "highly" before stringent conditions. Support for highly stringent can be found throughout the specification. For example, at page 10, lines 33 to page 11, line 2. The use of the term "highly stringent conditions" is clear with respect to its metes and bounds. Accordingly, the rejection with respect to 35 USC § 112, second paragraph should be withdrawn.

Claims 21 and 40-45

Claims 21 and 40-45 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the term "a bleomycin" of "a bleomycin analogue" is allegedly indefinite. As stated in the Action (dated December 4, 2002), "[t]he Examiner ha[s] questioned whether bleomycin is a single compound or a class of compounds." Action (dated December 4, 2002) at page 10. Applicants traverse. As stated in the specification, "[t]he bleomycins are a family of glycopeptide-derived antibiotics ...." *See*, specification at page 16, lines 28-30. For example, "[t]he commercial product, Blenoxane®, contains BLM A2 and B2 as the principle constituents." See, specification at page 16, line 33 to page 17, line 1. Thus, a bleomycin is a compound that belongs to the family of bleomycins, and a bleomycin analogue is an analogue of a member of the bleomycin family. As previously stated, the term analogue that is used claims 21 and 40-45 is term

well understood by those of skill in the art and one frequently allowed by the Patent Office in various claims. The use of the term "a bleomycin" and "a bleomycin analogue" reasonably conveys to one of skill in the art of the scope of the invention and is as precise as the subject matter permits. Accordingly, the rejection with respect to 35 USC § 112, second paragraph should be withdrawn.

Claims 40-45

Claims 40-45 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the antecedent basis for "a nucleic acid." Action (dated December 4, 2002) at page 10. Applicants have amended claim 21 to include a nucleic acid, which corrects the antecedent basis for claims 40-45. In addition, Applicants have amended claim 40 to include "the nucleic acid" to correct antecedent basis. According, the rejection with respect to 35 USC § 112, second paragraph should be withdrawn.

Claim 72

Claim 72 was rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the term "a resistance gene from the bleomycin gene cluster" is allegedly indefinite. Applicants have canceled claim 72. Thus, the rejection is moot and should be withdrawn.

Claims 5, 10 and 40-45

Claims 5, 10, and 40-45 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the language of claim 5 and 10 is allegedly unclear, seemingly due to typographical errors. Action (dated December 4, 2002) at page 15. Applicants traverse. Applicants have canceled claim 5; thus, the rejection is moot with respect to this claim. Applicants note that claim 9 and 10 are distinct. Claim 9 recites "a nucleic acid encoding a protein comprising the sequence of SEQ ID NO:115" while claim 10 recites "wherein the sequence of said protein is SEQ ID NO:115." Claim 9 uses the term "comprising" while claim 10 uses the term "is." Thus, claim 9 and claim 10 are not identical. Accordingly, the rejection with respect to claim 10 (and its dependent claims 40-45) should be withdrawn.

Claims 17 and 40-45

Claims 17, and 40-45 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the added limitation of encoding a module, when the parent claim is drawn to a specific polypeptide that is not part of the larger, polyketide synthase modular make-up is

allegedly unclear. Action (dated December 4, 2002) at page 15. Applicants have canceled claim 17; thus, the rejection with respect to claims 17 and 40-45 should be withdrawn.

For at least the reasons above, Applicants respectfully request that the rejections with respect to 35 U.S.C. §112, second paragraph be withdrawn.

35 U.S.C. §112, First Paragraph.

Claims 1-3, 21, 40-45 and 71-73 were rejected under 35 U.S.C. §112, first paragraph, as allegedly not meeting the written description requirement. Applicants traverse.

Claims 1-3, and 40-45

The Examiner noted "that if the rejection under 35 U.S.C. § 112, second paragraph is overcome for Claims 1-3, the written description rejection herein would also be obviated." Action (dated December 4, 2002) at page 12. Applicants have amended to claim 1, which should obviate the 35 U.S.C. § 112, second paragraph rejection. Amended claim 1 recites "highly stringent conditions." Thus, the rejection of claim 1 (and its dependent claims 2-3, 40-45) under 35 U.S.C. § 112, first paragraph should be withdrawn.

Claim 21 and 40-45

"The written description requirement does not require the applicant 'to describe exactly the subject matter claimed, [instead] the description must clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed.'" Union Oil Co. v Atlantic Richfield et al. 208 F.3d 989 (Fed. Cir. 2000) citing In re Gosteli, 872 F.2d 1008, 1012, 10 U.S.P.Q.2D (BNA) 1614, 1618 (Fed. Cir. 1989). Claim 21 recites "[a]n isolated gene cluster comprising a nucleic acid, which nucleic acid comprises open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin or a bleomycin analogue, wherein a polypeptide of the polypeptides is SEQ ID NO:115." Applicants have provided examples of an isolated bleomycin gene cluster. *See, e.g.,* the specification at page 3, lines 9-25, at page 5, lines 7-8, at page 19, line 10 to page 23, line 7, Example 1 at page 44, line 12 to page 54, line 25, etc. Applicants have also provided illustrations of modifications that can be made to the gene cluster to make bleomycin analogues. *See, e.g.,* at page 32, line 9-page 43, line 15. One of skill reading the specification would recognize that Applicants have invented what is claimed. The specification provides a written description of the claimed subject matter meeting the requirements of 35 U.S.C. § 112, first paragraph. Accordingly, the rejection should be withdrawn.

Claim 71-73

Claim 71 recites "[a] cell comprising a modified bleomycin gene cluster, wherein the modified bleomycin gene cluster comprises a nucleic acid which encodes a protein comprising SEQ ID NO:115, said cell producing elevated amounts of bleomycin as compared to the wild type cell." Applicants have provided examples of a cell overexpressing bleomycin. *See, e.g.,* page 56, lines 1-18. Thus, one of skill would recognize that Applicants have invented what is claimed by reading the specification. Accordingly, claims 71 and 73 meet the written description requirement of 35 USC § 112, first paragraph and the rejection with respect to these claims should be withdrawn. Claim 72 is canceled; thus, the rejection with respect to this claim should be withdrawn.

For at least the reasons above, Applicants respectfully request that the rejections with respect to 35 U.S.C. §112, first paragraph be withdrawn.

35 U.S.C. §102.

Claims 1, 40, 41, and 43-45 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Redenbach et al. ("Redenbach"). The Examiner alleges that Redenbach teaches a nucleotide sequence as encoding an oxygen-independent coproporhyrinogen III oxidase. Action (dated December 4, 2002) at page 14. In addition, in the action dated March 12, 2002 (at page 22), the Examiner alleged that Redenbach disclosed a nucleotide sequence that encodes a protein that is 34% identical to SEQ ID NO: 115 and that such a sequence will hybridize to ORF 8 under stringent conditions. Applicants traverse.

With respect to claims 1, 40, 41, and 43-45, in order for a reference to anticipate an invention, anticipation requires that "all limitations of the claim are found in the reference, or 'fully met' by it." Kalman v. Kimberly-Clark Corp., 218 USPQ 781, 789 (Fed. Cir. 1983). Amended claim 1 recites "[a]n isolated nucleic acid comprising a polynucleotide that hybridizes under highly stringent conditions to a SEQ ID NO:1, base pairs 57583-58854, wherein said polynucleotide encodes a protein that has an oxidase activity." All the limitations of amended claim 1 (and its dependent claims 40, 41 and 43-45) are not found in Redenbach. For example, Redenbach does not teach a nucleotide sequence encoding a protein with an oxidase activity. Table 2 of Redenbach, which list details of genetic markers assigned to the cosmid encyclopaedia, does not indicate a protein with an oxidase activity. If the Examiner feels that the rejection should be maintained, Applicants respectfully ask that the Examiner to point out where in the Redenbach publication a

nucleotide sequence encoding a protein with an oxidase is taught. Amended claim 1 recites "a polynucleotide that hybridizes under highly stringent conditions to a SEQ ID NO:1, base pairs 57583-58854." Redenbach does not list any specific sequence information. The sequences of Redenbach were submitted to GenBank on 17 January 2000, which is after the filing date of the above referenced application. Thus, the specific nucleotide sequences of Redenbach were not known as of the filing date of the application. Furthermore, amended claim 1 includes the limitation "highly stringent conditions." Redenbach does not teach a nucleotide sequence that would hybridize to SEQ ID NO: 115 under highly stringent conditions. Accordingly, Redenbach does not teach all the limitations of claim 1 and its dependent claims 40, 41, and 43-45. Redenbach does not teach specific nucleotide sequences, a nucleotide sequence that with hybridize to ORF 8 under highly stringent conditions or a nucleotide sequence that encodes a protein with oxidase activity. Accordingly, Applicants respectfully request that the rejection under 35 U.S.C. § 102(b) be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

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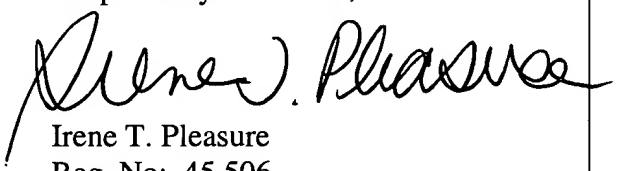
Respectfully submitted,



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APPENDIX A

"MARKED UP" CLAIMS ILLUSTRATING THE AMENDMENTS MADE TO THE CLAIMS OF 09/477,962 WITH ENTRY OF THIS AMENDMENT

1. (Twice amended) An isolated nucleic acid comprising a polynucleotide that hybridizes under highly stringent conditions to a SEQ ID NO:1, base pairs 57583-58854, wherein said polynucleotide encodes a protein that has an oxidase activity.

5. (Canceled) ~~The isolated nucleic acid of claim 1, wherein said the sequence of said protein is SEQ ID NO:115. nucleic acid comprises a nucleic acid encoding a protein encoded by a gene selected from the group consisting of blmI, blmII, and blmXI.~~

10. (Twice amended) The nucleic acid of claim 9, ~~wherein said nucleic acid~~ wherein the sequence of said protein is SEQ ID NO:115.

12. (Twice amended) The nucleic acid of claim 9, wherein said nucleic acid further comprises a nucleic acid encoding a protein encoded by ~~blmVIII~~SEQ ID NO:99.

13. (Twice amended) The nucleic acid of claim 9, wherein said nucleic acid further comprises a nucleic acid encoding a protein selected from the group consisting of ~~blmI~~SEQ ID NO:113, ~~blmII~~SEQ ID NO:109, and ~~blmXI~~SEQ ID NO:96.

14. (Twice amended) The nucleic acid of claim 9, wherein said nucleic acid further comprises a nucleic acid encoding a protein selected from the group consisting of ~~blmIII~~SEQ ID NO:107, SEQ ID NO:106~~blmIV~~, SEQ ID NO:102~~blmV~~, SEQ ID NO:101~~blmVI~~, SEQ ID NO:100~~blmVII~~, SEQ ID NO:98~~blmIX~~, and SEQ ID NO:97~~blmX~~.

15. (Cancel) ~~The nucleic acid of claim 9, wherein said nucleic acid further comprises blmVIII.~~

17. (Cancel) ~~The nucleic acid of claim 9, wherein said isolated nucleic acid comprises a nucleic acid encoding a module.~~

21. (Twice amended) An isolated gene cluster comprising a nucleic acid, which nucleic acid comprises open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin or a bleomycin analogue, wherein a polypeptide of the polypeptides is SEQ ID NO:115.

40. (Twice amended) An expression vector comprising ~~a~~ the nucleic acid of any one of claims 1, 2, 3, ~~5,~~ 9, 10, 12, 13, 14, ~~15, 17,~~ and 21.

71. A cell comprising a modified bleomycin gene cluster ~~nucleic acid~~, wherein the modified bleomycin gene cluster comprises a nucleic acid which encodes a protein comprising SEQ ID NO:115, said cell producing elevated amounts of bleomycin as compared to the wild type cell.

72. (Cancel) ~~The cell of claim 71, wherein said cell overexpresses a resistance gene from the bleomycin gene cluster.~~

73. (Twice amended) The cell of claim ~~72~~71, wherein said cell overexpresses a resistance gene from the bleomycin gene cluster and wherein said resistance gene is a selected from the group consisting of blmA~~,~~ and blmB.

APPENDIX B

CLAIMS PENDING IN USSN 09/477,962 WITH ENTRY OF THIS AMENDMENT

1. An isolated nucleic acid comprising a polynucleotide that hybridizes under highly stringent conditions to a SEQ ID NO:1, base pairs 57583-58854, wherein said polynucleotide encodes a protein that has an oxidase activity.

2. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least one additional open reading frame that encodes a polypeptide selected from the group consisting of SEQ ID NO:114, SEQ ID NO:113, SEQ ID NO:112, SEQ ID NO:111, SEQ ID NO:110, SEQ ID NO:109, SEQ ID NO:108, SEQ ID NO:107, SEQ ID NO:106, SEQ ID NO:105, SEQ ID NO:104, SEQ ID NO:103, SEQ ID NO:102, SEQ ID NO:101, SEQ ID NO:100, SEQ ID NO:99, SEQ ID NO:98, SEQ ID NO:97, SEQ ID NO:96, SEQ ID NO:95, SEQ ID NO:94, SEQ ID NO:93, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, and SEQ ID NO:126.

3. The isolated nucleic acid of claim 1, wherein said nucleic acid comprises a nucleic acid encoding at least two additional open reading frames encoding polypeptides independently selected from the group consisting of SEQ ID NO:114, SEQ ID NO:113, SEQ ID NO:112, SEQ ID NO:111, SEQ ID NO:110, SEQ ID NO:109, SEQ ID NO:108, SEQ ID NO:107, SEQ ID NO:106, SEQ ID NO:105, SEQ ID NO:104, SEQ ID NO:103, SEQ ID NO:102, SEQ ID NO:101, SEQ ID NO:100, SEQ ID NO:99, SEQ ID NO:98, SEQ ID NO:97, SEQ ID NO:96, SEQ ID NO:95, SEQ ID NO:94, SEQ ID NO:93, SEQ ID NO:116, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:119, SEQ ID NO:120, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:123, SEQ ID NO:124, SEQ ID NO:125, and SEQ ID NO:126.

9. An isolated nucleic acid comprising a nucleic acid encoding a protein comprising the sequence of SEQ ID NO:115.

10. The nucleic acid of claim 9, wherein the sequence of said protein is SEQ ID NO:115.

12. The nucleic acid of claim 9, wherein said nucleic acid further comprises a nucleic acid encoding a protein encoded by SEQ ID NO:99.

13. The nucleic acid of claim 9, wherein said nucleic acid further comprises a nucleic acid encoding a protein selected from the group consisting of SEQ ID NO:113, SEQ ID NO:109, and SEQ ID NO:96.

14. The nucleic acid of claim 9, wherein said nucleic acid further comprises a nucleic acid encoding a protein selected from the group consisting of SEQ ID NO:107, SEQ ID NO:106, SEQ ID NO:102, SEQ ID NO:101, SEQ ID NO:100, SEQ ID NO:98, and SEQ ID NO:97.

21. An isolated gene cluster comprising a nucleic acid, which nucleic acid comprises open reading frames encoding polypeptides sufficient to direct the assembly of a bleomycin or a bleomycin analogue, wherein a polypeptide of the polypeptides is SEQ ID NO:115.

40. An expression vector comprising the nucleic acid of any one of claims 1, 2, 3, 9, 10, 12, 13, 14, and 21.

41. A host cell transformed with an expression vector of claim 40.

42. The host cell of claim 41, wherein said cell is transformed with an exogenous nucleic acid comprising a gene cluster encoding polypeptides sufficient to direct the assembly of a bleomycin or bleomycin analog.

43. The cell of claim 41, wherein said cell is a bacterial cell.

44. The cell of claim 43, wherein said cell is a Streptomyces cell.

45. The cell of claim 41, wherein said cell is a eukaryotic cell.

71. A cell comprising a modified bleomycin gene cluster, wherein the modified bleomycin gene cluster comprises a nucleic acid which encodes a protein comprising SEQ ID NO:115, said cell producing elevated amounts of bleomycin as compared to the wild type cell.

73. The cell of claim 71, wherein said cell overexpresses a resistance gene from the bleomycin gene cluster and wherein said resistance gene is a selected from the group consisting of blmA and blmB.

APPENDIX C

"MARKED UP" PARAGRAPHS ILLUSTRATING THE AMENDMENTS MADE TO THE SPECIFICATION OF 09/477,962 WITH ENTRY OF THIS AMENDMENT

1. (Twice Amended) paragraph at page 15, lines 13-15

Figures 6A-6F illustrate the use of the *blm* NRPS and PKS enzymes to synthesize a variety of hybrid polyketide/peptide molecules including, but not limited to, a family of oxazolines/oxazoles, and thiazoline/thiazoles. Figure 6A synthesis using BlmIX, BlmVIII, and BlmVII. Figure 6B synthesis using NRPS, BlmVIII, and BlmVII. Figure 6C synthesis using BlmIX, BlmVIII, and BlmVII. Figure 6D synthesis using BlmIX, BlmVIII, and NRPS (C, A^N, PCP). Figure 6E synthesis using BlmIX, BlmVIII and NRPS (C, A^C, PCP). Figure ~~6E~~ 6F synthesis using BlmIX, BlmVIII, and NRPS (C, A^C, PCP, OX).

2. (Twice Amended) paragraph at page 19, lines 12-19

The nucleic acids comprising the *blm* gene cluster are identified in Tables I and II and listed in the sequence listing provided herein (SEQ ID NOS: 1 and 2, GenBank Accession numbers ~~AF149091,~~ AF210249 (which replaces sequence AF149091), and SEQ ID NO:3, GenBank Accession number AF210311). In particular, Table I identifies genes and functions of open reading frames (ORFs) responsible for the biosynthesis of the hybrid peptide/polyketide/peptide backbone and sugar moieties of bleomycin, while Table II identifies a number of ORFs comprising the *blm* gene cluster, identifies the activity of the catalytic domain encoded by the ORF and provides primers for the amplification and isolation of that orf.

3. Table 1 at pages 19-20

Table I. Determined functions of ORFs in the bleomycin biosynthesis gene cluster

Gene	Amino acids	Sequence Homolog[1]	Proposed function[2, 3]
orf8	424 SEQ ID NO: 115	YqeR (BAA12461)	Oxidase
orf9 (blmC)	498 SEQ ID NO: 114	RfaE (AAD07904)	NDP-glucose synthase
orf10 (blmI)	90	GrsB (P14688)	Type II PCP

	SEQ ID NO: 113		
orf11 (blmD)	545 SEQ ID NO: 112	NodU (Q53515	Carbamoyl transferase
orf12 (blmE)	390 SEQ ID NO: 111	RfaF (AAD16056)	Glycosyl transferase
orf13	187 SEQ ID NO: 110	MbtH (O05821)	Unknown
orf14 (blmII)	462 SEQ ID NO: 109	Nrp (CAA98937)	NRPS condensation enzyme
orf15	339 SEQ ID NO: 108	SyrP (1890776)	Regulation
orf 16 (blmIII)	935 SEQ ID NO: 107	HMWP2 (P48633), McbC (P23185)	A PCP Ox
orf17 (blmIV)	2626 SEQ ID NO: 106	HMWP2 (P48633)	C A PCP Cy A PCP Cy
orf18	638 SEQ ID NO: 105	AsnB (2293165)	Asparagine synthetase
orf19 (blmF)	494 SEQ ID NO: 104	RfbC (Q50864)/BlmOrf1 (507319)	Glycosyl transferase/β-hydroxylase
orf20 (blmG)	325 SEQ ID NO: 103	YtcB (2293288)	Sugar epimerase
orf21 (blmV)	645 SEQ ID NO: 102	McyB (2708278)	PCP C
orf22 (blmVI)	2675 SEQ ID NO: 101	ACoAS (1658531), PksD (S73014) SnbDE (CAA67249)	A^4 ACP C A PCP C A
orf23 (blmVII)	1218 SEQ ID NO: 100	SyrE (3510629)	C A PCP
orf24 (blmVIII)	1841 SEQ ID NO: 99	HMWP1 (CAA73127)	KS AT MT KR ACP
orf25 (blmIX)	1066 SEQ ID NO: 98	SafB (1171128)	C A PCP
orf26 (blmX)	~~2140~~2162 SEQ ID NO: 97	TycC (2623773)	C A PCP C A PCP
orf27 (blmXI)	688 SEQ ID NO: 96	SyrE (3510629)	NRPS condensation enzyme
orf28	239 SEQ ID NO: 95	SC9C7.04C (CAA22716)	Unknown
orf29	582 SEQ ID NO: 94	YvdB (CAB08068)	Transmembrane transporter
orf30	113 SEQ ID NO: 93	SmtB (P30340)	Regulation
orf31	117 SEQ ID NO: 116	PhnA (P16680)	Unknown

4. Table II, at pages 21-23.

Table II. *Blm* gene cluster open reading frames (ORFs) and primers for ORF amplification.

Orf #	~~Positio n~~	Activity	Method	Primers Forward Reverse	Seq ID No.
orf-8 SEQ ID NO:115	~~76183-77457~~	Oxygen-independent coproporphyrinogen III oxidase	Gapped-blast comparison[1]	F: ATGAGCCACGCCATCGGA R: TCAGGCGCGTTCGGGGGC	5 6
orf-9 SEQ ID NO:114	~~74690-76186~~	ADP-heptose synthase (*blmC*)	Gapped-blast comparison[1]	F: GTGAACACCGACCTGCCC R: TCATGGGGTGTCTCCCTC	7 8
orf-10 SEQ ID NO:113	~~74421-74693~~	Peptidyl carrier protein (*blmI*)	Expression and biochemical characterization.[2]	F: ATGAGCGCCCCGCGGGGC R: TCACCGGTCCCGCTCCCC	9 10
orf-11 SEQ ID NO:112	~~72787-74424~~	Carbamyltransferase (*blmD*)	Gapped-blast comparison[1]	F: ATGAGCGCCGACCCGTCC R: TCATGAGCGGGCCGCCGT	11 12
orf-12 SEQ ID NO:111	~~71618-72790~~	ADP-heptose:LPS heptosyl transferase (*blmE*)	Gapped-blast comparison[1]	F: ATGACCACCCCCATGACC R: TCATGGGGTACTCCTGAT	13 14
orf-13 SEQ ID NO:110	~~70983-71546~~	Homolog of mbtH in the synthesis of mycobactin	Gapped-blast comparison[1]	F: ATGACCACGACCCCGCGG R: TCAGGTGCCGGACACGCG	15 16
orf-14 SEQ ID NO:109	~~69598-70986~~	Peptide synthetase (condensation, *blmII*)	Gapped-blast comparison[1]	F: GTGACCGCCCCCGGCACA R: TCATCGGTGGCTCCTCGT	17 18
orf-15 SEQ ID NO:108	~~68582-69601~~	Regulatory gene (homolog of *syrP*)	Gapped-blast comparison[1]	F: GTGAACCGGCACGGCCCC R: TCACGCGCTCACCTCGTC	19 20
orf-16 SEQ ID NO:107	~~65778-68585~~	Mutated peptide synthetase- oxidase (NRPS-0, *blmIII*)	Gapped-blast comparison[1]	F: GTGACGAGCGCCCGGCCC R: TCACGGGGCCTCCGTGCG	21 22
orf-17 SEQ ID NO:106	~~57901-65781~~	Peptide synthetase (NRPS-2-1,*blmIV*)	Expression and biochemical characterization.[2]	F: ATGCTGCACGGCGCCGCG R: TCACTCCGGTCCACCTCC	23 24
orf-18 SEQ ID NO:105	~~55899-57815~~	Asparagine synthetase	Gapped-blast comparison[1]	F: GTGAGGCCCGTGTGCGGC R: TCAGCCACCGTTGCCGCC	25 26
orf-19 SEQ ID NO:104	~~54418-55902~~	Homolog of hydroxylase-dehydrogenase (*blmF*)	Gapped-blast comparison[1]	F: GTGAAGGACCTCGGCCGG R: TCACTCCCCCGGTGCCGG	27 28
orf-20 SEQ ID NO:103	~~53427-54404~~	Nucleotide-sugar epimerase (*blmG*)	Gapped-blast comparison[1]	F: GTGACATGGACCGTGGTG R: TCAGGCATCGGCCCTCCC	29 30
orf-21 SEQ ID NO:102	~~51493-53430~~	Peptide synthetase (NRPS-3CT, *blmV*)	Gapped-blast comparison[1]	F: ATGCGCGGGCATGACGAC R: TCACGGTGTCTCTCCCTC	31 32
orf-22 SEQ ID NO:101	~~43263-51290~~	Peptide synthetase (NRPS-5-4-3, *blmVI*)	Expression and biochemical characterization.[2]	F: ATGAGCCGGCCGGCCGGC R: TCATGCTCGGTCATCGCC	33 34
orf-23 SEQ ID NO:100	~~39610-43266~~	Peptide synthetase (NRPS-6, *blmVII*)	Expression and biochemical characterization.[2]	F: GTGACCACGCCCCGCATC R: TCATTCGGGACGCGGGCA	35 36
orf-24 SEQ ID NO:99	~~34088-39613~~	Polyketide synthase (*blmVIII*)	Gapped-blast comparison[1]	F: ATGAGCCATGCCGACGCG R: TCACAGCACCACCTCTTC	37 38
orf-25 SEQ ID NO:98	~~30891-34091~~	Peptide synthetase (NRPS-7, *blmIX*)	Gapped-blast comparison[1]	F: ATGACCCCGGCCGCCGAC R: TCATCGTCCGCCGCCTTT	39 40
orf-26 SEQ ID NO:97	~~24406-30894~~	Peptide synthetase (NRPS-9-8, *blmX*)	Gapped-blast comparison[1]	F: ATGCCTCGGTGTGCCCGA R: TCATTCGGCGGCACCTCC	41 42
orf-27 SEQ ID NO:96	~~22127-24193~~	Peptide synthetase (condensation, *blmXI*)	Gapped-blast comparison[1]	F: GTGGGTTTCCGTCGAGCG R: TTACACCCTCCGTTTCTC	43 44

orf-28 SEQ ID NO:95	~~21367-22086~~	Phosphatidylserine decarboxylase	Gapped-blast comparison[1]	F: ATGGCACAGGACCTGAAC R: TCAACGCCACCGGATCTT	45 46
orf-29 SEQ ID NO:94	~~19161-20909~~	Transmembrane transporter	Gapped-blast comparison[1]	F: GTGAGCTCCCTCGCCGTC R: TCATCGTCGGGCACTCGG	47 48
orf-30 SEQ ID NO:93	~~18823-19164~~	Metal dependent regulatory element	Gapped-blast comparison[1]	F: GTGCCGGTTCCGCTGTAT R: TCACCGGGCACTGACCTC	49 50
orf-31 SEQ ID NO:116	~~18660-18307~~	PHNA homolog	Gapped-blast comparison[1]	F: GTGACCGAGAACCTTCCG R: TCAGACCTTCTTGACCAC	51 52
orf-32 SEQ ID NO:117	~~17736-9211~~	Peptide synthetase (NRPS-11-10)	Gapped-blast comparison[1]	F: ATGGCCTCAGACGCTTTG R: TCATTGAGACTCCTCCTC	53 54
orf-33 SEQ ID NO:118	~~9214-7859~~	Putative transporter	Gapped-blast comparison[1]	F: ATGATGAAGTCAAGCCGC R: TCAGTGGCTTACAAGGAG	55 56
orf-34 SEQ ID NO:119	~~7797-6784~~	Homolog of clavaminic acid synthase	Gapped-blast comparison[1]	F: ATGACTGACCTGCCGTTG R: TCACACCAGCAGCGAGGT	57 58
orf-35 SEQ ID NO:120	~~6773-6021~~	Thioesterase	Gapped-blast comparison[1]	F: ATGGATTTCCCCCTCACC R: TCATGCCCCTACCTCGGC	59 60
orf-36 SEQ ID NO:121	~~6024-4741~~	Putative transporter	Gapped-blast comparison[1]	F: ATGACCGCGCGCGTCGAC R: TCACTCCTCGGCTTCGGC	61 62
orf-37 SEQ ID NO:122	~~4733-3915~~	Unknown	Gapped-blast comparison[1]	F: GTGTCCAAGAACGCGGCG R: TCATCGGCTCGCCTCGTG	63 64
orf-38 SEQ ID NO:123	~~3918-2182~~	Peptide synthetase (NRPS-12)	Gapped-blast comparison[1]	F: ATGACCCTCACCCTGCGG R: TCACTCGGGCACTCCTTC	65 66
orf-39 SEQ ID NO:124	~~2185-1199~~	Regulatory gene (homolog of *SyrP*	Gapped-blast comparison[1]	F: GTGACCGGTTCCGTAACG R: TCATGAGTCCGCCGAGGT	67 68
orf-40 SEQ ID NO:125	~~1015-1~~	Peptide synthetase	Gapped-blast comparison[1]	F: ATGACAGAGGTCCGAGGT R: CCCGGCAACCGCCCTCCC	69 70
orf-41 SEQ ID NO:126	~~On a separate sequence~~	4'-phosphopantetheinyl transferase (*pptA*)	Expression and biochemical characterization.[2]	F: GTGATCGCCGCCCTCCTG R: TTACGGGACGGCGGTCCG	71 72

5. paragraph on page 69, line 17 through page 70, line 20.

The sequence of the 1,761-bp *Bam*HI-*Sal*I fragment was analyzed for coding regions by using the CODONPREFERENCE and TESTCODE programs of the GCG package (Genetics Computer Group, Madison, Wisconsin). Two complete ORFs (*pptA, orf3*) and two incomplete ORFs (*orf1, orf4*) were identified within the sequenced region (Figure 13). The first ORF from left to right (designated *orf1*) starts out of the analyzed area and ends with a TGA codon at position 248 of the sequenced fragment. Comparison of the deduced product of *orf1* with proteins encoded by nucleic acids in databases showed similarities with Rv2795c from *Mycobacterium tuberculosis* (GenBank AL008967) and SC5A7. 22 from *S. coelicolor* (GenBank AL031107), both of unknown function. The second ORF, *pptA*, contains the sequence amplified by PCR and used for the cloning of this locus. It comprises 741 nucleotides, starting with a GTG codon (position 245) which is coupled to the stop codon of *orf1*, and ending with a TAA codon. The starting codon of *pptA* is

preceded by a potential ribosomal binding site (RBS), GGGAG. The overall (76. 6%) and third codon position (93. 9%) G+C contents and the codon usage of *pptA* are similar to those found in other *Streptomyces* genes, with the exception of the stop codon (TAA), which is most uncommon in this group of organisms (Wright et al. *Gene* (1992) 113:55-65). The *pptA* gene encodes a protein of 246 amino acids with a predicted molecular mass of 25,619 Da and a pI of 4. 76, which contains the conserved PPTase motifs. Databases searches with PptA showed significant similarities to the putative actinomycete PPTases (39-52%/48-61% identity/similarity) and to confirmed bacterial PPTases such as EntD from *E. coli* (17%/24% identity/similarity) (Lambalot et al. *Chem. Biol.* (1996) 3:923-936). The third ORF, *orf3*, is separated from *pptA* by an apparently noncoding DNA region of 153 bp, and it is transcribed in opposite and convergent direction with respect to *orf1-pptA*. The gene *orf3* comprises 240 nucleotides, starting with an ATG codon (position 1358) and ending with TGA. The starting codon of *orf3* is preceded by the sequence GAAGG, a potential RBS. The deduced product of *orf3* encodes a protein of 79 amino acids with a predicted mass of 7,555 Da and a pI of 7. 17. The Orf3 protein shows similarities to the N-terminal region of SC5H1. 35c, a protein of unknown function from *S. coelicolor* (encoded by nucleic acid sequence in GenBank AL049863). Analysis of Orf3 with the SignalP program (Nielsen et al. *Protein Engineer.* (1997) 10:1-6) predicts an N-terminal signal peptide which would be cleaved between residues 27 and 28 (ALA-DS), suggesting that the mature protein (52 amino acids, 5,099 Da, pI 4. 31) would be secreted. Between *orf3* and *orf4* there is an apparently noncoding region of 251 nucleotides. The *orf4* gene is transcribed in opposite and divergent direction with respect to *orf3*. It starts with an ATG codon at position 1610, preceded by a potential RBS (GGAGG), and ends out of the sequenced fragment. The deduced protein product (50 amino acids) of the incomplete *orf4* contains a potential NAD/FAD binding motif, $GXGX_2GX_3GX_6G$ (SEQ ID NO:92) (Scrutton et al. *Nature* (1990) 343:38-43), showing low similarities to diverse oxidoreductases.